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## **Role of microRNAs in stem/progenitor cells and cardiovascular repair**

Jakob, P ; Landmesser, U

**Abstract:** MicroRNAs (miRNAs), small non-coding RNAs, play a critical role in differentiation and self-renewal of pluripotent stem cells, as well as in differentiation of cardiovascular lineage cells. Several miRNAs have been demonstrated to repress stemness factors such as Oct4, Nanog, Sox2 and Klf4 in embryonic stem cells, thereby promoting embryonic stem cell differentiation. Furthermore, targeting of different miRNAs promotes reprogramming towards induced pluripotent stem cells. MicroRNAs are critical for vascular smooth muscle cell differentiation and phenotype regulation, and miR-143 and miR-145 play a particularly important role in this respect. Notably, these miRNAs are down-regulated in several cardiovascular disease states, such as in atherosclerotic lesions and vascular neointima formation. MicroRNAs are critical regulators of endothelial cell differentiation and ischaemia-induced neovascularization. miR-126 is important for vascular integrity, endothelial cell proliferation and neovascularization. miR-1 and miR-133 are highly expressed in cardiomyocytes and their precursors and regulate cardiomyogenesis. In addition, miR-499 promotes differentiation of cardiomyocyte progenitor cells. Notably, miRNA expression is altered in cardiovascular disease states, and recent studies suggest that dysregulated miRNAs may limit cardiovascular repair responses. Dysregulation of miRNAs may lead to an altered function and differentiation of cardiovascular progenitor cells, which is also likely to represent a limitation of autologous cell-based treatment approaches in these patients. These findings suggest that targeting of specific miRNAs may represent an interesting novel opportunity to impact on endogenous cardiovascular repair responses, including effects on stem/progenitor cell differentiation and functions. This approach may also serve to optimize cell-based treatment approaches in patients with cardiovascular disease.

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# Role of microRNAs in stem/progenitor cells and cardiovascular repair

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## Abstract

microRNAs (miRNAs), small non-coding RNAs, play a critical role in differentiation and selfrenewal of pluripotent stem cells as well as in differentiation of cardiovascular lineage cells. Several miRNAs have been identified to repress stemness factors such as Oct4, Nanog, Sox2 and Klf4 in embryonic stem cells, thereby promoting embryonic stem cell (ESC) differentiation. Furthermore, targeting of different miRNAs promotes reprogramming towards induced pluripotent stem cells (iPSCs).

miRNAs are critical for vascular smooth muscle cell (VSMC) differentiation and phenotype regulation, and miR-143 and miR-145 play a particularly important role in this respect. Notably, these miRNAs are downregulated in several cardiovascular disease states, such as in atherosclerotic lesions and vascular neointima formation. miRNAs are critical regulators of endothelial cell differentiation and ischemia-induced neovascularisation. miR-126 is important for vascular integrity, endothelial cell proliferation and neovascularisation. miR-1 and miR-133 are highly expressed in cardiomyocytes and their precursors and regulate cardiomyogenesis. In addition, miR-499 promotes differentiation of cardiomyocyte progenitor cells.

Notably, miRNA expression is altered in cardiovascular disease states, and recent studies suggest that dysregulated miRNAs may limit cardiovascular repair responses. Dysregulation of miRNAs may lead to an altered function and differentiation of cardiovascular progenitor cells, likely also representing a limitation of autologous cell-based treatment approaches in these patients.

These findings suggest that targeting of specific miRNAs may represent an interesting novel opportunity to impact on endogenous cardiovascular repair responses, including effects on stem/progenitor cell differentiation and functions. This approach may also serve to optimize cell-based treatment approaches in patients with cardiovascular disease.

**This article is part of the Review Focus on: The Role of MicroRNA in Cardiovascular Biology and Disease**

## Introduction

microRNAs (miRNAs) are small, non-coding RNAs regulating gene expression at the post-transcriptional level by mRNA degradation or translational repression.<sup>1</sup> The human genome has been estimated to contain up to 1000 miRNAs.<sup>1</sup> The regulation and processing of miRNAs is still incompletely understood, however, important progress has been made in unravelling of these pathways over the past several years. In the first part of this review article we are summarizing some recent insights into the biogenesis and processing of miRNAs (see also Figure 1), that are also important for the discussion of studies evaluating the role of miRNAs in stem/progenitor cell differentiation and function.

Notably, miRNAs have been identified to target transcription factors critically regulating embryonic stem cell self-renewal and differentiation.<sup>2-5</sup> In addition, several miRNAs have been shown to facilitate reprogramming towards induced pluripotent stem cells.<sup>6-10</sup> Moreover, specific miRNAs have been identified to regulate differentiation and function of cardiac, vascular smooth muscle and endothelial progenitor cells, which are discussed in the present article.

Furthermore, emerging data suggest that in cardiovascular disease altered miRNA expression may limit and impair cardiovascular repair responses, including differentiation and function of stem/progenitor cells. Hence, understanding of the physiological and pathophysiological regulation and role of miRNAs in stem and progenitor cells will lead to an improved understanding of cardiovascular disease pathophysiology and likely provide interesting novel potential therapeutic targets to promote endogenous cardiovascular repair processes.

## miRNA biogenesis and processing

Most miRNAs are located within introns, and are processed from the primary transcript of their host genes.<sup>11</sup> This co-transcription of miRNAs is thought to aid in the fine-tuning of biological processes regulated by the host genes.<sup>12-14</sup> miRNAs are mostly transcribed by RNA polymerase II,<sup>15</sup> that generates the primary transcript with a stem-loop structure, called pri-miR (Figure 1).

pri-miRs are further processed by the microprocessor, i.e. the complex of the nuclear RNase III protein Drosha and the associated co-factor DGCR8 (DiGeorge syndrome critical region 8), which releases a shorter hairpin of about 65 nucleotides (pre-miR) after cleavage.<sup>16, 17</sup> Pre-miRs are transported via exportin-5 from the nucleus to the

cytoplasm,<sup>18</sup> where they are cleaved by Dicer, a RNase III enzyme.<sup>19-23</sup> One strand of the unwound miRNA helix is preferentially chosen and loaded into the RNA-induced silencing complex (RISC), whereas the other strand, miRNA\*, is degraded.<sup>24, 25</sup> In the RISC, miRNAs interact mainly with the 3' untranslated regions (UTRs) of mRNAs, leading to mRNA destabilization or translational repression or both.<sup>26</sup> Recent data suggest that in humans miRNAs largely lead to mRNA destabilization.<sup>27</sup> Of note, more recently miRNAs have also been suggested to increase protein expression of some targets,<sup>28, 29</sup> however, the underlying mechanisms need to be further determined.

miRNA biogenesis may also differ from the above canonical stepwise processing pathway. For example, mirtrons are very short introns of genes that by splicing directly form pre-miRNAs and are therefore not processed by Drosha.<sup>30, 31</sup> In addition, there are miRNAs that are processed independent of Dicer that require the Argonaute protein Ago2 for miRNA maturation.<sup>32, 33</sup>

### **miRNAs regulate embryonic stem cell differentiation and self-renewal**

*Dicer and DGCR8-knockout inhibit ESC differentiation:* The contribution of miRNAs to differentiation and function of embryonic stem cells (ESCs) has initially been elucidated using knockout models of parts of the miRNA-processing pathways, i.e. deletion of Dicer or the Drosha-cofactor DGCR8. Notably, Dicer-deficiency was lethal at an early stage of development in mouse embryos.<sup>34</sup> In vitro, Dicer-null ESCs failed to differentiate and remained in a self-renewal state, indicating a critical role of miRNAs for ESC differentiation.<sup>35</sup> Similarly, DGCR8-knockout ESCs failed to undergo cell lineage commitment, and had an incomplete silencing of the pluripotency factors Oct4, Sox2, Nanog and Rex1.<sup>36</sup> These findings underscore the critical role of miRNAs for ESC differentiation into specific cell lineages (Figure 2).

*miRNAs regulating ESC self-renewal and differentiation:* Subsequently, several miRNAs have been observed to be preferentially expressed in embryonic stem cells. By sequencing short RNAs, the miRNA 290-295 cluster has been found to be highly expressed in embryonic stem cells.<sup>2</sup> These miRNAs promote ESC proliferation by facilitating the G1-S transition in the cell cycle,<sup>37, 38</sup> and are therefore considered to have a role in maintaining ESC pluripotency (Figure 2).

Several miRNAs have been identified to be part of the ESC differentiation process and repress multiple pluripotency factors involved in self-renewal of ESCs as described below. MiR-134, miR-296 and miR-470, which are upregulated in the differentiation of mouse embryonic stem cells, target the transcription factors Nanog, Sox2 and Oct4.<sup>4</sup> miR-21 suppresses self-renewal of mouse ESCs, at least in part by decreasing expression of the pluripotency factors Oct4, Nanog, Sox2 and c-Myc.<sup>3</sup> miR-145 expression is low in self-renewing human ESCs and is highly upregulated during human ESC differentiation. miR-145 directly targets the pluripotency factors Oct4, Sox2, and Klf4 and increased miR-145 expression inhibits ESC self-renewal, represses expression of the above pluripotency genes, and induces lineage-restricted differentiation.<sup>5</sup>

### **Targeting miRNAs to promote reprogramming towards induced pluripotent stem cells**

Mouse fibroblasts were the first differentiated somatic cells, which were reprogrammed into iPSCs by viral transduction of Oct3/4, Sox2, c-Myc, and Klf4.<sup>39</sup> Human fibroblasts could subsequently be converted into iPSCs by transduction of the same four transcription factors<sup>40</sup> or by introduction of Oct4, Sox2, Nanog, and Lin28.<sup>41</sup> As mentioned above, stemness factors are regulated by miRNAs, which renders them highly interesting for promoting nuclear reprogramming processes of somatic cells, given the advantage of a nonviral transfection procedure. The role of miRNAs in promoting reprogramming of iPSCs has therefore been intensely examined.<sup>42</sup>

*Enhancing transcription factor induced reprogramming towards iPSCs using miRNAs:* Notably, silencing of members of the let-7 miRNA family in murine somatic cells has been shown to promote reprogramming towards induced pluripotent stem cells (iPSCs).<sup>8</sup> Introduction of miR-291-3p, miR-294 and miR-295 together with the transcription factors Oct4, Sox2 and Klf4 substantially enhanced the efficacy of reprogramming towards iPSCs.<sup>7</sup> These miRNAs have been termed ES cell-specific cell cycle-regulating miRNAs (ESCC) because of their abundance and role in proliferation of mouse ESCs.<sup>37</sup> Similar results have been reported using the human ESCC miRNA orthologs miR-302b and miR-372.<sup>10</sup>

*Induction of reprogramming towards iPSCs using miRNAs without stemness factors:*

Recently, a non-transcription factor mediated methodology using miRNAs to reset mouse embryonic fibroblasts into a pluripotent state has been reported.<sup>6</sup> By transducing the miR-302/367 cluster as well as the Hdac-inhibitor VPA into mouse embryonic fibroblasts an increased efficacy of iPSC formation was observed as compared to transduction using stemness factors (with VPA).<sup>6</sup>

Moreover, it has been shown that it is possible to reprogram cells to pluripotency without viral transfection by direct transfection of mature miRNAs (miR-200c, miR-302 s, and miR-369s family members) into adipose stromal cells (mASCs).<sup>9</sup> However, the efficacy of dedifferentiation was reduced as compared to the viral transfection procedures, especially in human dermal fibroblasts.<sup>9</sup>

The above studies demonstrate that miRNAs are strongly involved in the regulation of pluripotency and differentiation of stem cells. Lineage commitment steps are frequently regulated by multiple miRNAs. Targeting miRNAs has therefore become an interesting novel approach to modulate stem cell differentiation. A further refined understanding of these miRNA-regulated pathways will lead to novel tools to guide stem cell differentiation and function that is particularly important for regenerative medicine. Moreover, direct reprogramming of adult fibroblasts into induced cardiomyocytes has recently been reported,<sup>43</sup> and it will be highly interesting to see whether this can also be enhanced or even achieved alone by targeting or introduction of miRNAs.

**miRNAs regulating vascular smooth muscle cell differentiation and phenotype**

*VSMC specific knockout of Dicer reveals a critical role of miRNAs in VSMC differentiation:* Recently, the role of miRNAs in differentiation and function of VSMCs has been elucidated by generating a VSMC-specific Dicer knockout mouse. Mutant mice died between embryonic day 16 and 17 due to extensive hemorrhage and a thinned wall of the aorta caused by a decreased proliferation of VSMCs.<sup>44</sup> Furthermore, a loss of contractile function and differentiation of VSMCs could be observed, whereas transfection with mimic miR-145 could partially rescue the impaired contractile function and differentiation of VSMCs.<sup>44</sup>

*miR-143 and miR-145 regulate VSMC differentiation and phenotype:* MiR-143 and miR-145 are co-transcribed and are crucial for the VSMC cell fate, i.e. regulation of

differentiation, phenotype and function, of vascular smooth muscle cells (VSMC).<sup>29</sup> These miRNAs are co-transcribed and are controlled by Nkx2-5 (NK2 transcription factor related, locus 5) and the SRF (serum response factor) with its co-activator Myocardin. miR-143 promotes VSCM differentiation by repressing Elk-1,<sup>29</sup> and thereby preventing Elk-1 dependent suppression of VSMC differentiation marker gene expression.<sup>45</sup> The potent properties of miR-145 to promote VSMC differentiation were underlined by the observation that overexpression of miR-145 in neural crest stem cells resulted in differentiation towards VSMCs in vitro.<sup>29</sup> VSMCs of mice lacking either miR-143, miR-145 or both are expressing a less differentiated phenotype of VSMCs, indicating that miR-143/145 modulate the fine-tuning of the plasticity of VSMCs.<sup>46</sup> Consistently, a decrease of contractile VSMCs and an accumulation of synthetic VSMCs were found in the aorta and the thinned femoral artery in another study examining a miR-143/miR-145 knockout mouse model.<sup>47</sup> Moreover, agonist-mediated contractility in arterial rings and blood pressure in vivo were reduced in miR-143/145-knockout mice.<sup>47</sup>

Inducible depletion of SMC-specific Dicer in adult mice led to a similar, although substantially more severe phenotype as observed in miR-143/135 knockout mice, suggesting that additional miRNAs are involved in maintaining postnatal VSMCs in a differentiated state.<sup>48</sup>

*miR-1 regulates VSMC differentiation:* Recently, miR-1, which mediates cardiac muscle differentiation after activation of Myocardin, was shown to be involved in the differentiation process of VSMCs.<sup>49, 50</sup> Overexpression of Myocardin in human VSMCs induces miR-1 and represses proliferation. This process can be reversed by inhibition of miR-1.<sup>49</sup> Furthermore, ESC differentiation into VSMCs depends on miR-1. miR-1 expression was increased during differentiation of ESCs into VSMCs, whereas inhibition of miR-1 represses VSMC differentiation.<sup>50</sup> miR-1 shares an identical target with miR-145, the transcription factor Klf4.<sup>50</sup>

*miR-221 and miR-222 regulate VSMC phenotype:* miR-221 and miR-222 target the cyclin-dependent kinase (CDK) inhibitors p27 and p57 in VSMCs and thereby promote cell cycle progression and proliferation of these cells.<sup>51, 52</sup> Notably, knockdown of miR-221 and miR-222 suppressed VSMC proliferation in vivo and



neointimal lesion formation after carotid angioplasty,<sup>52, 53</sup> and these miRNA may therefore provide a potential therapeutic target to suppress neointima formation.<sup>51, 52</sup>

### **miRNAs regulate vascular integrity, endothelial cell proliferation and function**

*Dicer knockdown reveals critical role of miRNAs in vascular development and endothelial cell functions:* Several miRNAs have been identified to play an important role in vascular growth, endothelial cell differentiation, proliferation and functions. First attempts to gain insight into the role of miRNAs in vascular development/angiogenesis were made by engineering vertebrates lacking Dicer.<sup>20</sup> Dicer-deficiency resulted in lethality in early development.<sup>34, 53</sup> Morphological analysis revealed less developed blood vessels in embryos and fewer blood vessels in yolk sacs, suggesting that compromised vessel formation/maintenance is leading to embryonic lethality.<sup>53</sup> Consistent with this observation, mice with a hypomorphic Dicer expression are infertile owing to an impaired angiogenesis in the corpus luteum.<sup>54</sup> Moreover, a reduction of endothelial miRNAs by Cre-dependent knockout of Dicer in ECs resulted in an impaired angiogenesis in distinct models of post-natal angiogenesis.<sup>55</sup>

Subsequently, in vitro studies using Dicer-deficient endothelial cells (EC) were performed. Silencing of Dicer in endothelial cells caused an impairment of capillary sprouting, tube formation and migration capacity.<sup>56, 57</sup> This was, at least in part, related to downregulation of pro-angiogenic miRNAs, i.e. let-7f and miR-27b, and a subsequently increased expression of several anti-angiogenic factors.<sup>57, 58</sup>

*miR-126 regulates vascular integrity, endothelial cell proliferation and neovascularisation:* The most abundant miRNA in CD31<sup>+</sup> cells is miR-126.<sup>59</sup> Morpholino-induced knock-down of miR-126 in zebrafish caused compromised vascular integrity and led to hemorrhages.<sup>59</sup> Similarly, defects in vascular maintenance were also shown in mice lacking miR-126.<sup>60</sup> 40% of miR-126 deficient mice died embryonically or perinatally due to defects in endothelial cell proliferation, migration, and angiogenesis causing leaky vessels and hemorrhages.<sup>60</sup> The surviving subset of mutant mice had a markedly increased mortality and reduced infarct borderzone neovascularisation after myocardial infarction.<sup>60</sup> In summary, miR-126 is important for maintaining vessel integrity during embryogenesis. This was further highlighted in a study investigating aortic arch development in zebrafish,

where blood flow activated miR-126, which in turn enhanced vascular endothelial growth factor (VEGF) signaling, thereby facilitating angiogenic sprouting.<sup>61</sup> However, miR-126 overexpression in ESCs failed to upregulate endothelial cell specific markers in vitro, hence it is not sufficient to induce endothelial lineage commitment in ESCs.<sup>59</sup>

*miR-17-92 cluster regulates neovascularisation:* As first shown in several studies in the oncology field, the miR-17-92 cluster, which is induced by the transcription factor c-Myc, regulates distinct angiogenic functions.<sup>62, 63</sup> miR-92a, a member of the miR-17-92 cluster, exerts antiangiogenic functions.<sup>64</sup> Antagomir-based silencing of miR-92a increases infarct borderzone neovascularisation after myocardial infarction.<sup>64</sup> MiR-17, miR-18a, miR-19a, and miR-20a inhibit angiogenic activity of mature endothelial cells in vitro and knockdown of miR-17 and miR-20a increased vessel formation as observed in a matrigel plug assay in vivo.<sup>65</sup> In contrast, miR-17-5p, miR-18a, and miR-20a promote proliferation and cord formation in Dicer-deficient human ECs<sup>55</sup> and enhance neovascularisation in tumor cells transduced with the miR-17-92 cluster,<sup>66</sup> indicating that the regulatory mechanisms of the miR-17-92 cluster differ in distinct tissues and are depending on the cellular context. Therefore, the effect of miRNAs from the 17-92 cluster differs for ischemia-induced and tumor-associated angiogenesis, which is important if one considers these miRNAs as potential therapeutic targets.

### **miRNAs and cardiomyocyte differentiation**

*miR-1 and miR-133 control cardiomyocyte differentiation:* miR-1 and miR-133 are highly expressed in cardiac and skeletal muscle cells and their precursors, belong to the same transcriptional unit and play a critical role in myogenesis.<sup>67-70</sup> miR-1, consisting of 2 miRNA genes (miR-1-1 and miR-1-2) is co-transcribed with 2 (miR-133a-2 and miR-133a-1) out of 3 existing miR-133 genes.<sup>67, 70</sup> These miRNAs are induced by the muscle differentiation factors SRF, Mef2 and Myocardin.<sup>67, 71</sup>

miR-1 (and to a lesser extent miR-133) promotes mesodermal progenitor formation and suppresses non-muscle gene expression in mouse and human ESCs.<sup>72</sup> The miR-1 dependent adoption of a myogenic phenotype is supported by the repression of Hdac4, which negatively regulates Mef2, an essential transcription factor for the differentiation pathway of muscle cells.<sup>71, 73</sup> Furthermore, miR-1 downregulates Notch

ligand Delta-like 1 (DLL-1), which is involved in cardiomyocyte differentiation of ESCs and cardiac progenitor cell division.<sup>72</sup> MiR-1 also modulates the fine-tuning of cell polarity.<sup>74</sup> Fly embryos deficient for dmiR-1 had misaligned cardioblasts.<sup>74</sup>

In vivo, deletion of dmiR-1, the single ortholog of miR-1 in *Drosophila*, is lethal due to a loss of muscle differentiation.<sup>68</sup> miR-1-2 mutant mice revealed ventricle septum defects, cardiac hyperplasia and abnormal conduction.<sup>75</sup> In this study, miR-1-1, the second member of the miR-1 family, could not compensate for the loss of its redundant miR-1-2.<sup>75</sup> Overexpression of dmiR-1 decreased cardiac cell number<sup>68</sup> and cardiac-specific excess of miR-1 in mice decreased proliferation by targeting Hand2, a transcription factor regulating ventricular cardiomyocyte expansion.<sup>67</sup>

miR-133 plays a distinct role after mesodermal differentiation. In contrast to miR-1, miR-133 represses cardiac markers, promotes proliferation of myoblasts and inhibits differentiation through repression of SRF.<sup>72</sup> Depletion of the miR-133 family resulted in defects of cardiac morphogenesis and increased proliferation of cardiomyocytes, whereas single depletion resulted in a normal phenotype.<sup>76</sup>

*miR-17-92 cluster regulates cardiac development:* The miR-17-92 cluster contributes to regulation of heart development, as indicated by ventricular septal defects of the hearts in a loss-of-function model.<sup>77</sup> Furthermore, miR-92 controls endoderm development.<sup>78</sup> Overexpression of miR-92 in zebrafish results in a suppression of endoderm formation, which leads to cardia bifida.<sup>78</sup>

*miR-499 promotes cardiomyocyte differentiation:* During cardiomyocyte differentiation of human cardiomyocyte progenitor cells (hCMPC),<sup>79</sup> human cardiac stem cells (hCSC)<sup>80</sup> and human ES cells,<sup>81</sup> miR-499 is highly upregulated. Overexpression of miR-499 in human cardiomyocyte progenitor cells indirectly promotes cardiac muscle differentiation by targeting sex determining region Y-box 6 (Sox6) and regulator of differentiation 1 (Rod1) in vitro.<sup>79, 80</sup> Furthermore, overexpression of miR-499 in hESCs and hCMPCs upregulated cardiac marker expression, suggesting a role for miR-499 to control cardiomyocyte cell fate.<sup>79, 81</sup> However, in adult mice cardiac miR-499 overexpression resulted in cellular cardiac hypertrophy and cardiac dysfunction.<sup>82</sup>

### **miRNA alterations in human cardiovascular disease**

Dysregulated expression of miRNAs has been observed in human cardiovascular disease, including altered regulation in progenitor cells.<sup>83</sup> miRNA tissue profiling approaches in cardiomyopathies<sup>84</sup>, and in progenitor cells<sup>85</sup> revealed an altered regulation of miRNAs in different cardiovascular disease states. Subsequently, circulating human miRNA-expression profiles in plasma samples were investigated in distinct cardiovascular diseases such as stable coronary artery disease<sup>86</sup> and essential hypertension<sup>87</sup> as well as in patients with cardiovascular risk factors such as Diabetes mellitus.<sup>88, 89</sup>

Recently, miRNAs have been implicated in the functional cardiovascular repair capacity of progenitor cells (Table 1). Thus, differential miRNA regulation may play a role in cardiovascular disease by both, altering the repair potential of progenitor cells as well as by impacting on the function and phenotype of differentiated cells. Here we will discuss in particular miRNAs that may also play a role in progenitor cells.

### **miRNA regulation in early endothelial progenitor cells in cardiovascular disease**

Early endothelial progenitor cells (EPCs) have been suggested to promote endothelial cell growth (largely in a paracrine manner) and may thereby promote endothelial repair responses and improve cardiac function. As described in detail below, several recent studies have described alterations in miRNAs in early EPCs derived from patients with cardiovascular disease, that may critically limit the endogenous repair response.

*miR-21 is increased in early EPCs in coronary artery disease:* In circulating early endothelial progenitor cells (EPCs), miR-21 has been reported to be upregulated after treatment with asymmetrical dimethylarginine (ADMA).<sup>85</sup> miR-21 expression was increased in early EPCs from patients with coronary artery disease.<sup>85</sup> Moreover, overexpression of miR-21 in early EPCs has been shown to inhibit their migratory capacity through repression of superoxide dismutase 2 (SOD2), a key protection protein against oxidative damage, and the endogenous mitogen-activated protein kinase inhibitor sprouty-2 (SPRY2).<sup>85</sup>

*miR-221 and miR-222 are increased in early EPCs in coronary artery disease:* miR-221/222 levels have been observed to be increased in early EPCs derived from patients with coronary artery disease (CAD) as compared to healthy subjects and were inversely correlated with EPC number in these patients.<sup>90</sup> After treatment with Atorvastatin, miRNA-221/222 expression decreased and early EPC number increased in patients with CAD, but no causal relationship has been examined.<sup>90</sup> miR-221 and miR-222 are also expressed in VSMCs and ECs. ECs overexpressing miR-222 showed a diminished proliferation and vessel formation in matrigel plugs.<sup>91</sup> In VSMCs, platelet-derived growth factor (PDGF), a major growth factor to promote neointima formation, induces miR-221/222 expression in vitro.<sup>52</sup> Knockdown of miR-221/222 decreases cell proliferation in cultured VSMCs as well as neointima formation in balloon-injured rat arteries.<sup>52</sup>

*miR-126 and miR-130a are reduced in early EPCs in chronic heart failure:* Early EPCs from patients with chronic heart failure due to ischemic cardiomyopathy (ICM) showed a markedly reduced expression of miR-126 as compared to early EPCs from healthy subjects (HS), and the in vivo cardiac repair capacity of EPCs from healthy subjects was markedly reduced after anti-miR-126 transfection.<sup>92</sup> Moreover, a reduced expression of miR-130a was observed in early EPCs from patients with chronic heart failure.<sup>92</sup> This miRNA is known to promote angiogenesis by targeting the homeobox genes GAX and HOXA5 in ECs.<sup>93</sup>

The role of miR-126 in ischemia-induced angiogenesis has been further shown by an antagomir-based silencing of miR-126 in a hindlimb ischemia model, in which capillary vessel formation was reduced.<sup>94</sup>

*miR-34a limits pro-angiogenic capacity of early EPCs:* miR-34a has been suggested as an antiangiogenic and proapoptotic miRNA expressed in early EPCs, and has been first shown to target silent information regulator 1 (Sirt1) in colorectal carcinoma cells.<sup>95</sup> Sirt1 is a regulator of apoptosis, cell cycle and senescence and acts as a mediator of angiogenesis in ECs. Introduction of mimic miR-34a into rat early EPCs inhibited angiogenesis and induced senescence in vitro.<sup>96</sup> This was suggested to be mediated by Sirt1-targeting and partially by upregulation of a downstream target of Sirt1, forkhead box O transcription factor 1 (FoxO1).<sup>96</sup>

### **miRNAs mediate preconditioning and regulate viability of stem/progenitor cells**

Recent studies, as described below, have explored the role of miRNAs in mediating preconditioning effects on stem/progenitor cells. These findings may provide interesting novel opportunities for optimization of cell-based treatment approaches for cardiac repair.

*miR-21 exerts cytoprotective effects in skeletal myoblasts:* In skeletal myoblasts, miR-21 was upregulated after pharmacological preconditioning with Diazoxide.<sup>97</sup> The observed cytoprotective effects (with respect to oxidative stress challenge) were reduced after anti-miR-21 treatment, suggesting a critical role of miR-21 in this process.<sup>97</sup> miR-21 is also upregulated in left ventricles of rats after ischemic preconditioning in vivo and has been proposed to exert antiapoptotic effects through inhibition of programmed cell death 4 (PDCD4).<sup>98</sup>

*miR-146a protects MSCs from apoptosis:* Preconditioning of mesenchymal stem cells (MSCs) with Diazoxide prevented these cells from apoptosis through a NF- $\kappa$ B-dependent induction of miR-146a in vitro.<sup>99</sup> Upregulation of miR-146a conducts cytoprotective effects by repression of Fas (CD 95), a TNF receptor family-member, which is required for cell death programming.<sup>99</sup>

*miR-155 may improve survival of cardiomyocyte progenitor cells:* miR-155 is upregulated in human cardiomyocyte progenitor cells after exposure to hydrogen-peroxide and has been suggested to improve their survival and inhibit necrosis through repression of receptor interacting protein 1 (RIP1), a death domain receptor-associated protein.<sup>100</sup>

*miR-210 improves survival of transplanted MSCs in the heart:* Ischemic preconditioning of mesenchymal stem cells (MSCs) upregulated miR-210 expression in a Hif-1 $\alpha$ -dependent fashion and improved their survival after engraftment in the rat infarcted heart.<sup>101</sup> This was mediated by targeting FLASH/caspase-8-associated protein-2, a pro-apoptotic regulator.<sup>101</sup>

*miRNA cocktail (miR-21, miR-24, miR-221) improves viability of transplanted cardiac progenitor cells:* As miRNAs can improve the functional behavior of progenitor cells,

transfection with multiple miRNAs are an emerging approach in order to target the same deleterious transcript more efficiently or repressing multiple mRNAs in order to influence a pathophysiological process. In this respect, Hu et al. recently used a miRNA cocktail consisting of miR-21, miR-24 and miR-221 to transfect mouse cardiac progenitor cells, thereby showing that the viability of the transfected cells is enhanced after intramyocardial delivery, accompanied by an enhanced cardiac function after myocardial infarction.<sup>102</sup>

## Conclusions

miRNAs have been identified as critical regulators of embryonic stem cell self-renewal and differentiation. Several miRNAs have also been reported to promote iPSC reprogramming, which is particularly important for regenerative medicine. Specific miRNAs have been suggested to play a critical role in the differentiation of cardiovascular lineage cells, i.e. in vascular smooth muscle, endothelial cell and cardiomyocyte differentiation and function.

Notably, miRNAs are differentially regulated in different cardiovascular diseases, including miRNAs expressed in stem/progenitor cells. Altered expression profiles in circulating progenitor or resident tissue stem/progenitor cells may impair the endogenous cardiovascular repair capacity. As miRNA-targeted therapies are entering the clinical arena a detailed understanding of the role of miRNAs in stem/progenitor cell differentiation and functions as well as the alterations in cardiovascular disease is therefore required. A further understanding of the role of specific miRNAs in regulating or limiting endogenous cardiovascular repair responses involving stem/progenitor cells and cardiovascular lineage differentiation may lead to interesting novel therapeutic strategies to promote cardiovascular repair.

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**Disclosures**

None.



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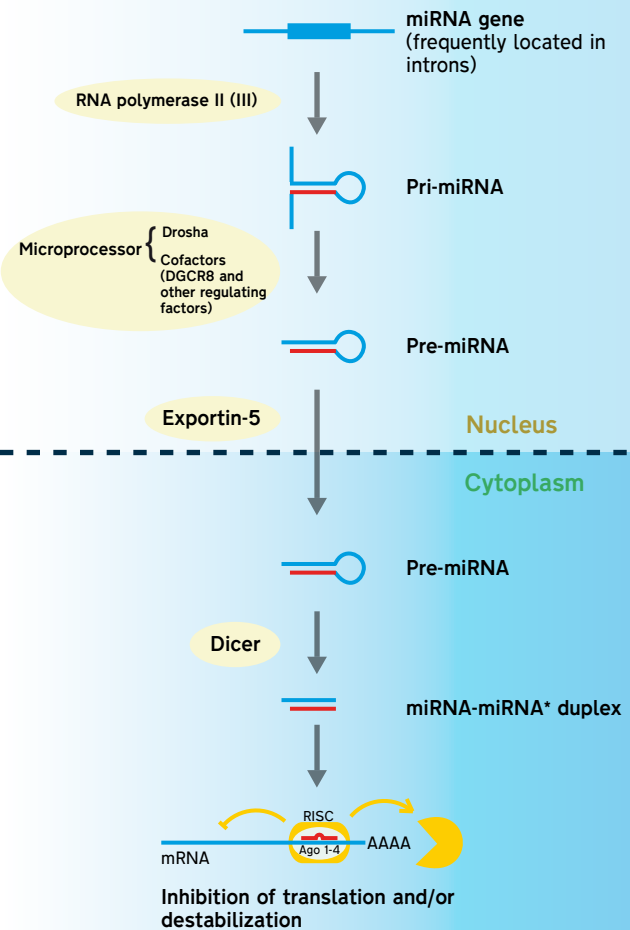
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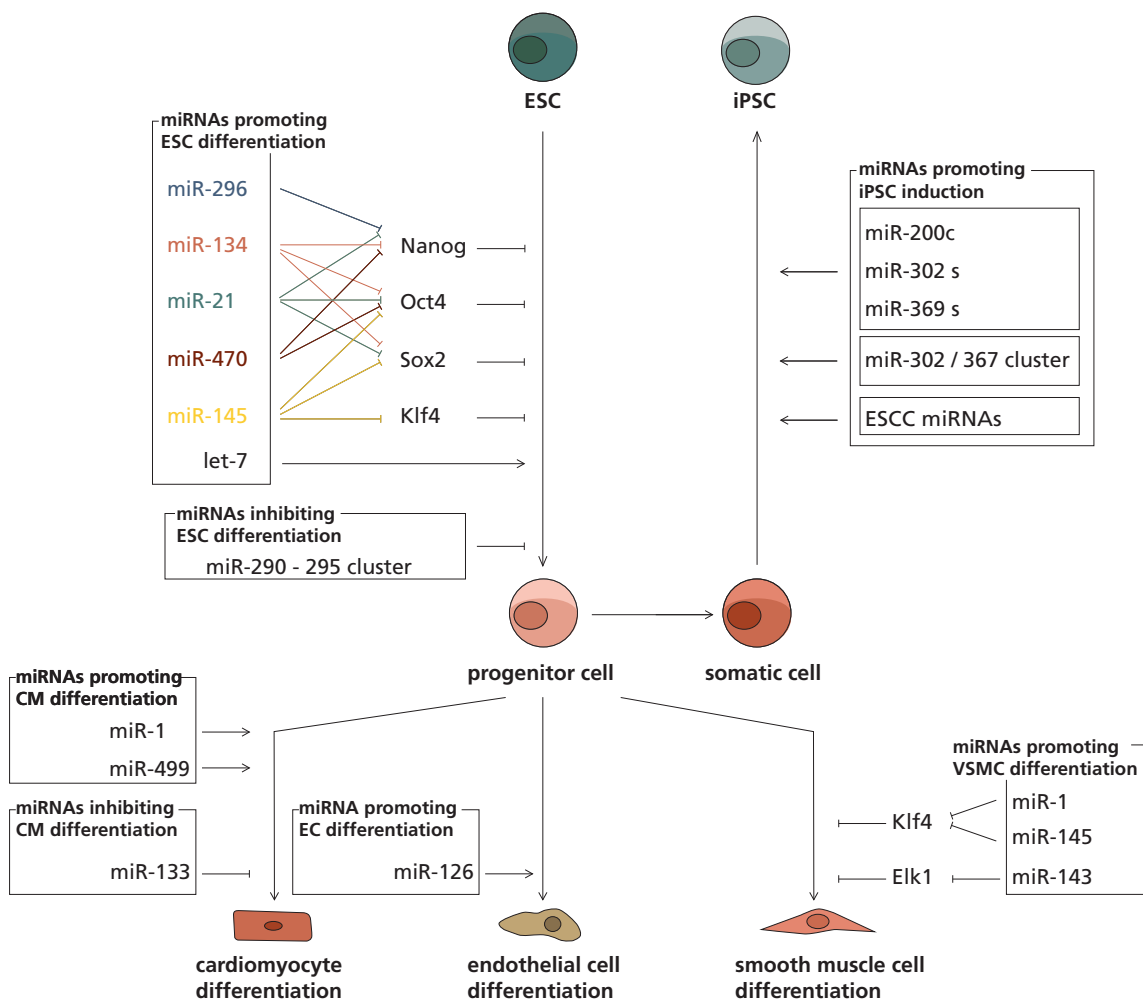


## Figure legend

**Figure 1: Biogenesis of miRNA:** Schematic of the canonical pathway of miRNA transcription and processing. miRNAs are transcribed mainly by RNA polymerase II leading to the primary transcript (pri-miRNA). In the nucleus, pri-miRNAs are cleaved by the RNA-specific endoribonuclease Drosha (and its cofactor DGCR8) resulting in the pre-miRNA. Exportin-5 transports the pre-miRNA into the cytoplasm. In the cytoplasm pre-miRNAs are processed by Dicer leading to the miRNA-miRNA\* duplex, where the miRNA is the active component and miRNA\* gets degraded. In the RNA-induced silencing complex (RISC) the miRNA strand leads to mRNA destabilization or translational repression of mRNA. RISC, RNA-induced silencing complex; Ago1-4, Argonaute 1-4

**Figure 2: *Role of miRNAs in self-renewal and differentiation of pluripotent stem cells and in the differentiation of stem/progenitor cells into cardiovascular lineage cells.*** Various miRNAs promote ESC differentiation, mostly by targeting stemness factors. On the other hand, the miR-290-295 cluster inhibits ESC differentiation. A subset of the miR-290-295 cluster (miR-291a-3p, miR-291b-3p, miR-294 and miR-295) plus miR-302 are termed ES cell-specific cell cycle-regulating miRNAs (ESCC) because they promote cell cycle progression in ESCs. These miRNAs have also been used to increase efficacy of iPSC formation. Furthermore, several miRNAs play an important role in cardiovascular cell lineage differentiation and the regulation of the phenotype of these cells. ESC, embryonic stem cell; iPSC, induced pluripotent stem cell; CM, cardiomyocyte; EC, endothelial cell; VSMC, vascular smooth muscle cell







Cell type	Differentially expressed miRNA	Disease/Modulator	Targets	Function	ref.
<b>A: miRNA regulation in early endothelial progenitor cells</b>					
Early endothelial progenitor cells	miR-21↑	CAD / ADMA	SOD2↓; SPRY2↓	Migration ↓ ROS formation↑	85
Early endothelial progenitor cells	miR-221/222↑	CAD		Early EPC number↓	90
Early endothelial progenitor cells	miR-126↓	CHF	Spred1↑	Angiogenesis↓	92
Early endothelial progenitor cells	miR-130a↓	CHF	HoxA5↑	Angiogenesis↓	92
Early endothelial progenitor cells	miR-34a↑	miRNA-overexpression	Sirt1↓	Senescence↑ Angiogenesis↓	96
<b>B: miRNAs mediating preconditioning and regulating viability of stem/progenitor cells</b>					
Mesenchymal stem cells	miR-210↑	IP	Flash/Casp8ap2↓	Apoptosis↓	101
Skeletal myoblast	miR-21↑	PP (Diazoxide)		Apoptosis↓	97
Mesenchymal stem cells	miR-146a↑	PP (Diazoxide)	Fas (CD95)↓	Apoptosis↓	99
Cardiomyocyte progenitor cells	miR-155↑	H <sub>2</sub> O <sub>2</sub>	RIP1↓	Necrosis↓	100
Cardiac progenitor cells	miR-21, miR-24, miR-221↑ (cocktail)	miRNA-overexpression	Bim↓	Viability↑	102

**Table 1:** miRNAs differentially regulated in early endothelial progenitor cells (EPCs) or miRNAs mediating preconditioning and regulating viability of stem/progenitor cells

CAD, coronary artery disease; ADMA, asymmetrical dimethylarginine; CHF, chronic heart failure; IP, ischemic preconditioning; PP, pharmacological preconditioning; SOD2, superoxide dismutase type II; SPRY2, Sprouty2; Spred1, Sprouty-related EVH-1 domain containing-1; Flash/Casp8ap2, FLICE-associated huge protein/caspase-8-associated protein-2; RIP1, receptor interacting protein 1; Sirt1, Silent information regulator 1; Bim: Bcl2l11 (Bcl-2 protein family)